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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : G01N 33/68 — A2

(11) International Publication Number: WO 00/05586

(43) International Publication Date: 3 February 2000 (03.02.00)

(21) International Application Number: PCT/US99/16802

(22) International Filing Date: 22 July 1999 (22.07.99)

(30) Priority Data:
09/120,435 22 July 1998 (22.07.98) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 09/120,435 (CIP)
Filed on 22 July 1998 (22.07.98)

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(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: A SUBFAMILY OF RNA HELICASES WHICH ARE MODULATORS OF THE FIDELITY OF TRANSLATION TERMINATION AND USES THEREOF

(57) Abstract

This invention provides a method of modulating translation termination efficiency of mRNA and/or promoting degradation of abberant transcripts. Also, this invention provides a method of screening for a drug active involved in enhancing translation termination and a method for identifying a disease state involving defective the protein complex. This invention provides a purified complex comprising an amount of MTT1, human Upf1p, a peptidyl eucaryotic release factor 1 (eRF1) and a peptidyl eucaryotic release factor 3 (eRF3) effective to modulate translation termination. Further, this invention provides an expression vector which comprises a nucleic acid encoding a MTT1, a human Upf1p protein, a peptidyl eucaryotic release factor 1 (eRF1) and a peptidyl eucaryotic release factor 3 (eRF3) operably linked to a regulatory element. This invention provides an antibody which binds to the complex comprising an amount of a MTT1, human Upf1p protein, a peptidyl eucaryotic release factor 1 (eRF1) and a peptidyl eucaryotic release factor 3 (eRF3) effective to modulate translation termination. This invention provides an agent which inhibits or modulates the binding of MTT1 to eRF3. The agent may inhibit or facilitate the binding of MTT1 to eRF3.

delineated by the first AUG codon encoding the amino acid methionine. After initiation of translation, the ribosome manufactures the polypeptide by progressing along the mRNA in the 5' to 3' direction, decoding one codon at a time. The final step in the translation process occurs when one of three termination codons occupies the A-site of the ribosome, resulting in hydrolysis of the peptide reviewed in Buckingham et al., 1997).

Although translation termination normally occurs after completion of the full-length polypeptide, base substitutions and frameshift mutations in DNA often lead to the synthesis of an mRNA that contains an inappropriate stop codon within its protein coding region. The occurrence of such a premature stop codon arrests translation at the site of early termination and causes the synthesis of a truncated protein and rapid degradation of the mRNA (reviewed in Ruiz-Echevarria et al., 1996; Weng et al., 1997). Interestingly, nonsense and frameshift mutations cause approximately 20-40% of the individual cases of over 240 different inherited diseases (reviewed in McKusick, 1994). Thus, treatment of a number of genetic disorders can be envisioned by promoting nonsense suppression. Nonsense suppression results when a near cognate tRNA successfully competes with the termination factors at a nonsense mutation so that amino acid incorporation into the peptide chain occurs rather than prematurely terminating translation (Fig. 1). Sufficient levels of nonsense suppression allows production of completed polypeptide protein. For many diseases in which only one percent of the functional protein is produced, patients suffer serious disease symptoms, whereas boosting expression to only five percent of normal levels can greatly reduce the severity or eliminate the disease (McKusick, 1994; Cooper etc.). Recent reports have demonstrated that sub-inhibitory concentrations of certain aminoglycosides suppress the translation termination process, resulting in the expression of full-length CFTR and restoring cyclic AMP-activated chloride channel activity (Bedwell et al. 1997; Howard et al., 1996). Thus, identifying and characterizing the factors that regulate the efficiency of the translation termination will be important for understanding the biology of this process as well as in developing therapeutics for the

This invention provides a method of identifying genes which are involved in modulation of the fidelity of translation termination, which comprises: a) isolated a gene of interest; and b) determining whether the gene of interest comprises motifs I-IX, wherein if the gene comprises any one of the nine motifs the gene modulates translation termination. In one embodiment motif I comprises the sequence: GppGTKTxT-X(n). In another embodiment

motif II comprises the sequence riLxcaSNxAvDxl-X(n).In another embodiment motif III comprises the sequence vviDExxQaxxxxxiPi- X(n).In another embodiment motif IV comprises the sequence xxi1 aGDxxQLp- X(n).In another embodiment motif V comprises the sequence lxx SLF erv- X(n).In another embodiment motif VI comprises the sequence LxxQYRMhpxisefpxYxgxL- X(n).In another embodiment motif VII comprises the sequence IgvitPYxxQvxxl- X(n).In another embodiment motif VIII comprises the sequence vevxtVDxFQGreKdxIilSc VR- X(n).In another embodiment motif IX comprises the sequence iGFLxdxRRINValTRak.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. 5 yeast proteins define a subclass of superfamily group I helicases. The MTT1, UPF1, DIP1, SEN1 and DNA2 helicase domains were aligned using PILEUP and the results plotted using BOXSHADE in the GCG program. The consensus sequence is listed on the bottom line. Conserved identical residues (dark gray box) are indicated by capital letters, while conserved similar residues are indicated by lowercase letters (light gray box). Amino acid number within the primary sequence of the respective genes is indicated in the figure.

FIGURE 2 An *mtt1*Δ demonstrates nonsense suppression. MTT1, UPF1, or MTT1 and UPF1 were deleted from yeast strain KC2 (ura3-52 trp1D leu2-2 tyr7-1) and these cells were grown to OD_{600}=1.0. Serial dilutions of these cells were plated on -ura-leu-tyr to assay for nonsense suppression, and -ura as

a control for cell growth. Growth was monitored at 30°C and 10 days growth is pictured above.

FIGURE 3 Mtt1 is not required for nonsense mediated mRNA decay. UPF1, or MTT1 and UPF1 were deleted from yeast strain KC2 (ura3-52 trp1D leu2-2 tyr7-1) and these cells were grown to OD_{600}=0.8. Total RNA was prepared and subjected to RNA blotting analysis, using a probe for CYH2 mRNA.

FIGURE 4 Mtt1 interacts withe eRF3. Cytoplasmic extracts from a yeast strain BJ3505 transformed with either pG-1 (vector) or pG-1FLAGMTT1 (Flag-Mtt1p) were prepared in IBTB and incubated with 30 μl GST, GST-eRF1, GST-eRF3, GST-eRF3NM or GST-eRF3C sepharose-protein complexes. The sepharose-protein complexes were washed 2 times in IBTB (see materials and methods), resuspended in SDS-PAGE loading buffer, separated on an 8% SDS-PAGE gel and immunoblotted using anti-FLAG antibody.

FIGURE 5 Mtt1 is polysome associated. Cytoplasmic extracts from a yeast strain BJ3505 transformed with pG-1FLAGMTT1 were prepared and either treated with RNAse A or left untreated. Extracts were then centrifuged through a 7-47% sucrose gradient. Gradients were harvested and fractions were collected while monitoring A_{254}. Gradient fractions were subjected to western blotting using monoclonal antibody to the Flag epitope as a probe. A_{254} profiles are shown in the top panels while western blots of the corresponding fractions are shown in the bottom panels.

DETAILED DESCRIPTION OF THE INVENTION

(eRF3), wherein the complex is effective to modulate peptidyl transferase activity.As defined herein a "surveillance complex" comprises at least MTT1, Upf1p; and eucaryotic Releasing Factor 1 and 3. The *"UPF1"* gene, is also called RENT1 or HUPF1. The complex may also comprise Upf2p and /or Upf3p.

This invention provides an agent which binds to the complex which modulates the fidelity of translation termination. Translation termination includes initiation, elongation, termination and degradation. In one embodiment the agent modulates the binding of MTT1 to the polysome. In another embodiment the agent inhibits the binding of human MTT1 to eRF3. In another embodiment the agent facilitates the binding of MTT1 to eRF3.

The results presented here demonstrated that the purified Mtt1p also shows RNA-dependent ATPase and helicase activities (Fig. 7). Several lines of evidence suggest that Mtt1p is involved in translation termination The results presented here show that; 1) a *mtt1*Δ strain demonstrates a nonsense suppression phenotype (Fig. 4); 2) the Mtt1p is polysome associated (Fig. 6); 3) the Mtt1p directly interacts with the peptidyl release factor eRF3 (Fig. 5); 4) *mtt1*Δ strains demonstrate paromomycin sensitivity. If one considers that, unlike a *upf1*Δ strain, a *mtt1*Δ strain does not stabilize nonsense-containing transcripts, then the amount of nonsense suppression per RNA molecule is greater in a *mtt1*Δ strain than in a *upf1*Δ strain (Fig. 3).

A large number of observations point to an important role for protein synthesis in the mRNA decay process. In fact, it appears that these two processes have co-evolved and that factors essential for one process also function in the other. Evidence for this linkage includes experiments demonstrating that: a) drugs or mutations that interfere with translational elongation promote mRNA stabilization, b) sequence elements that dictate rapid mRNA decay can be localized to mRNA coding regions and the activity

Results

Identification of a family of yeast superfamily group I helicases that are similar to the *UPF1* gene: A sequence comparison to identify other yeast genes that are homologous to the *UPF1* was undertaken and the results are shown in Figure 1. The *SEN1* gene demonstrated significant homology with *UPF1* (Fig. 2; see Koonin, 1992). *SEN1* was identified in a screen for mutations that affect tRNA splicing and harbors all of the motifs to be considered a superfamily group I helicase (Winey and Culbertson, 1988, DeMarini et al. 1992). The previously identified *DNA2* gene also demonstrated significant homology to *UPF1* (Fig. 2). DNA2 is likely to have a role in DNA replication, possibly in processing Okazaki fragments (Budd et al. 1995, Budd and Campbell 1997). Two additional genes encoding superfamily group I helicases with high homology to *UPF1* were also identified and in previous studies have been named Helicase A (*HCSA*, Biswas et al. 1997a,b) and Helicase B (*HCSB*, Biswas et al. 1995) or scHel1 (Bean and Matson, 1993). For reasons that will be described below, the gene encoding *Helicase B (HCSB)*, is named *MTT1* (for *M*odulator of *T*ranslation *T*ermination). The proteins encoded by the *HCSA* and *MTT1* genes have been previously purified and demonstrated to have DNA-dependent helicase activity (Biswas et al., 1995; Biswas et al., 1997a,b; Bean and Matson, 1993). HCSA and HCSB have been suggested to be involved in chromosome replication (Biswas et al., 1995, 1997a,b). This notion is based on the observations that; 1) the yeast single-stranded DNA binding protein Rpa1p enhances their DNA helicase activities (Biswas et al., 1995,1997) and 2) HcsA co-purified with DNA polymerase α, and displays the biochemical properties of replicative helicases (Biswas et al., 1997a,b). To date there is no *in vivo* evidence that suggests the involvement of *HCSA* or *HCSB* in replication. Both *hcsa*Δ and *hcsb*Δ strains are viable. *hcsb*Δ strains do not display defects in growth, sensitivity to DNA damage, or respiratory defects (Bean and Matson, 1997). Transposon insertion into the promoter region of *MTT1* has been reported to cause hypersensitivity to calcofluor white, a cell wall synthesis inhibitor and hygromycin B, a drug which induces translational misreading (Lussier et al. 1997). Homology of HcsA and HcsB has been noted previously (Biswas et al. 1997a).

The homology among these five yeast helicases appears to be confined to their helicase domains (Fig. 2).

Eight conserved motifs are associated with all superfamily group I helicases (Gorbalenya, 1988, Koonin, 1992). Within these eight motifs, a limited number of residues is conserved among all superfamily group I helicases. Although these 8 motifs are spaced variably from protein to protein, according to the crystal structure of 2 different superfamily group I helicases, these conserved residues are all in close proximity in 3 dimensions (2 crystal structure papers). A more careful analysis of the genes with similarity to *UPF1* identifies this group as a subclass of superfamily group I which, the UPF1-like subclass. The distinguishing feature of this subclass is a more extensive homology surrounding the conserved residues in motifs II, IV, V and VI (Fig. 2) which has been noted previously (Perlick et al. 1996). Furthermore two additional motifs within this domain are conserved among these five genes. The first is located between motifs III and IV (consensus lexSLFervl, fig. 2) and the second is located between motifs IV and V (consensus IgvitpYxaQ; Fig. 2), refered as motif IIIa and IVa, respectively. These additional motifs are present in the human homolog of the Upf1 gene as well. Of these five yeast genes, Dna2p is the poorest fit to the consensus, and omission of this sequence yields a tighter consensus. Two other superfamily group I helicases from yeast, Pif1 and RadH, and two well characterized group I helicases from *E. coli*, Rep and uvrD, could not be aligned to these five sequences under these parameters, indicating that the homology is not general to all superfamily group I helicases, thus evidence for a distinct subclass.

As described above, a unique feature of the Upf1p is that it contains a cysteine-histidine-rich region near it amino terminus (Fig. 2C). Mutations in this region have been shown to reduce translation termination efficiencies at nonsense codons and enhance programmed -1 ribosomal frameshifting efficiencies (Weng et al., 1996b; Cui et al., 1996). This region has been identified as the Upf2 interaction domain (Weng et al,

1996b, He et al. 1996). Interestingly, the Mtt1p also contains a cysteine-histidine rich region near its amino terminus (Bean and Matson, 1997). Within the first 127 amino acids, 13 cysteines and 3 histidines are present. Although the cysteine-histidine rich regions of *UPF1* and *MTT1* contain no apparent homology, both regions have the potential to form ring fingers (see Weng et al., 1996b, Bean and Matson, 1997). Furthermore these regions can be matched to multiple zinc-binding motifs. However, due to the considerable number of cysteine residues, any alignment of this type leaves several cysteine residues unaccounted for within the same region.

A *mtt1*Δ strain demonstrates a nonsense suppression phenotype:. Nonsense suppression results when a near cognate tRNA successfully competes with the termination factors at a nonsense mutation so that amino acid incorporation into the peptide chain occurs rather than prematurely terminating translation (Fig. 1). Sufficient levels of nonsense suppression allows production of completed polypeptide protein which can support growth. A *upf1*Δ strain allows nonsense suppression of these alleles. Based on these observations, it was determined that Mtt1p is involved in modulating translation termination at a stop codon. To test this possibility, wild-type, *mtt1*Δ, *upf1*Δ, *upf1*Δ *mtt1*Δ strains harboring *leu2-2* and *tyr7-1* nonsense alleles were assayed for suppression of these alleles. The suppression phenotype of strains harboring the *leu2-2* and *tyr7-1* nonsense alleles was monitored by plating cells on -trp -leu -tyr media. As a control, these cells were plated on -trp media. The results demonstrated that the both *upf1*Δ and *mtt1*Δ cells harboring grew on both types of media (Fig. 3A), indicating that deleting either the *UPF1 or MTT1* genes allowed suppression of the *tyr7-1* and *leu2-1* nonsense alleles (Fig. 3A). Wild-type (*UPF1*$^{+}$ *MTT1*$^{+}$) cells were unable to grow on -trp -leu -tyr media, demonstrating that the presence of these genes prevented suppression of these nonsense alleles (Fig. 3A).

The nonsense suppression phenotype of a *upf1*Δ *mtt1*Δ strain was also monitored as described above and compared to strains harboring single deletions. The results from

these experiments demonstrated that a *upf1*Δ *mtt1*Δ strain was much more effective in suppressing the *tyr7-1* and *leu2-1* nonsense alleles than strains harboring single deletions of either the *UPF1* or *MTT1* gene (Fig. 3A). Taken together, these results demonstrate that both the Upf1p and Mtt1p is involved in modulating translation termination at nonsense codons.

Previous results demonstrated that a *upf1*Δ strain was also able to enhance frameshift suppression at 37°C in strains harboring the *his4-38* allele and a *SUF1* tRNA frameshift suppressor while strains harboring the wild-type *UPF1* gene could not grow at this temperature (Leeds et al., 1991, 1992). It was shown that a *mtt1*Δ *his4-38 SUF1* strain would also be able to enhance frameshift suppression. The results demonstrated that, unlike a *upf1*Δ strain, *mtt1*Δ *his4-38 SUF1* strain was unable grow on media lacking histidine at 37°C, indicating that deleting the *MTT1* gene did not increase frameshift suppression in this assay.

A *mtt1*Δ strain does not affect nonsense-mediated mRNA decay: Previous results demonstrated that the Upf1p has a role in regulating both mRNA turnover and translation termination (Weng et al. 1996a,b, 1998; Czaplinski et al., 1998). Based on these results, it was determined whether the nonsense suppression phenotype observed above was a consequence of affecting the efficiency of translation termination, inactivating the nonsense-mediated mRNA decay pathway (NMD), or a combination of affecting the two pathways. A *mtt1*Δ strain harboring the *tyr7-1* and *leu2-2* nonsense containing alleles was constructed to ask this question (see Experimental Procedures). A *mtti*Δ strain was shown to be viable with no demonstrable growth defects (See Experimental Procedures). The effect of a *mtt1*Δ on NMD was examined by monitoring the abundance of the CYH2 precursor and mature mRNA, which encodes a ribosomal protein. The inefficiently spliced CYH2 precursor, which contains an intron near the 5' end, is a naturally occurring substrate for the nonsense-mediated mRNA decay (NMD) pathway (He et al., 1993). The abundance of the nonsense-containing tyr7 and leu2 transcripts was also determined in

these strains. The results demonstrated that the steady-state levels of CYH2 precursor and mature mRNA, the tyr7 and leu2 mRNAs were equivalent to that found in a wild-type strain (Fig. 3). As a control, the CYH2 precursor and nonsense-containing tyr7 and leu2 transcripts were increased in a *upf1*Δ strain. The abundance of the wild-type CYH2 transcript, which is not a substrate of the NMD pathway, was equivalent in all strains tested (Fig. 3). Furthermore, the abundance of the transcripts that are substrates for the NMD pathway are not affected any greater in a *upf1*Δ *mtt1*Δ strain versus only a *upf1*Δ strain (Fig. 3). A *upf1*Δ *mtt1*Δ strain was viable with no discernable growth defects.

The Mtt1p interacts with the peptidyl release factor eRF3: Previous results suggested that the Upf1p affects translation termination by directly interacting with the translation termination factors eRF1 and eRF3 and therefore may affect their efficiency of translation termination (Czaplinski et al., 1998). Since deleting the *MTT1* gene also promotes nonsense suppression of the *tyr7-1* and *leu2-2* alleles, Mtt1p also interacts with the peptidyl release factors. To test this, eRF1 and eRF3 were individually expressed in *E. coli* as glutathione-S-transferase (GST) fusion proteins and purified using glutathione sepharose beads. The purified GST-RF (release factor) fusion proteins associated with the glutathione sepharose beads were added to a yeast cytoplasmic extract containing a FLAG epitope-tagged Mtt1p (see Experimental Procedures). Following incubation, the GST-RFs and associated proteins were purified by affinity chromatography and subjected to SDS-PAGE. Immunoblotting was performed and the presence of the Flag-Mtt1p was assayed using an antibody against the FLAG epitope. The anti-FLAG antibody recognized only the 127 kD Mtt1p in cytoplasmic extracts from cells transformed with plasmid expressing the FLAG-Upf1p. This analysis also demonstrated that the Mtt1p specifically co-purified with eRF3 (Fig. 5). Mtt1p did not co-purify with either GST-RF1 or GST protein that was not fused to another protein (Fig.5) or a GST-JIP protein, in which a Jak2 interacting protein fused to GST was used to monitor the specificity of the reaction.

The Mtt1p is polysome-associated: Based on nonsense suppression phenotypes of a *mtt1*Δ strain, it was investigated whether the Mtt1p is associated with ribosomes. To determine whether the Mtt1p is ribosome-associated, post-mitochondrial extracts were prepared from cells harboring the Flag-*Mtt1* gene and the polysome fractions were separated by centrifugation through sucrose gradients. The various fractions were collected and the presence of the Flag-Mtt1p protein in the gradient fractions were determined by Western blotting, probing the blots with an antibody directed against the Flag epitope. The results from these experiments indicated that the Flag-Mtt1p is polysome- and monosome-associated while the upper fractions contained no detectable Mtt1p (Fig. 6 lanes 2 and 3). The Mtt1p associated with the polysome fraction consists predominantly of an 127 kD protein. Treatment of the polysome extracts with RNase A shifted the Mtt1p to fractions which contain the 40 S subunits.

The Mtt1p demonstrates RNA-dependent ATPase and helicase activities: Previous results have shown that Mtt1p has DNA-dependent ATPase and helicase activities (Biswas et al., 1995). Based on the results described above, it was shown that Mtt1p also will be an RNA-dependent ATPase and helicase. It was first asked whether purified Mtt1p exhibited ATPase activity. ATPase assays were performed by incubating the purified protein in reaction mixtures containing radiolabelled [γ-^{32}P]ATP in the presence or absence of a poly-uridine (poly(rU)) and assaying the release of $^{32}PO_4$. The results demonstrated that in the absence of poly(rU) no ATPase activity was detectable (Figs. 7). Reaction mixtures containing poly(rU), however, greatly stimulated the release of $^{32}PO_4$, indicating that Mtt1p also harbored an RNA-dependent ATPase activity (Fig. 7). Concentrations of poly(rU) at or above 330nM maximally stimulated the ATPase activity of the Upf1p.

Discussion

The results presented here describe the identification of Mtt1p, a nucleic acid dependent helicase with significant homology to the Upf1p, a factor previously identified in regulating both translation termination and NMD (Czaplinski et al., 1995,1998; Weng

et al., 1996a,b, 1998). Several lines of evidence indicate that Mtt1p also has a role in modulating the translation termination process. Interestingly, comparison of the *MTT1* gene with other superfamily group I helicases identified unique signature motifs that tag this subfamily of superfamily group I helicases as possibly being involved in either RNA dependent or RNA-DNA dependent processes (Fig. 2). As will be discussed below, these results suggest that a subset of the Upf1p family of RNA helicases are involved in modulating the efficiency of the translation termination process.

The *MTT1* gene and its protein product demonstrate similarity to the Upf1p: A comparison of the *MTT1* and *UPF1* genes identified several regions of similarity. Both proteins contain a cysteine-histidine rich region near the amino terminal end of the protein and harbor all of the motifs to be a superfamily group I helicase (Fig.2). The cysteine-histidine rich regions of *UPF1* and *MTT1* are not very homologous. It is also conceivable that these cysteine-histidine-rich regions form a new type of cysteine-histidine-rich motif. Interestingly, mutations in the cysteine-histidine rich region of *UPF1* have been shown previously to increase programmed -1 frameshifting efficiencies and promote nonsense suppression (Cui et al., 1996; Weng et al., 1996b).

Sequence comparisons of superfamily group I helicases initially identified *SEN1* and *MOV-10* genes as having strong regions of homology to the *UPF1* gene helicase region (Koonin, 1992). *MTT1* gene also demonstrates extensive homology to *UPF1* and to other members of the *UPF1*-like subfamily (Fig. 2). These genes include *DNA2*, *HCSA*, *HCSB/MTT1*, and *SEN1*. In particular, another member of the *UPF1* family of helicases is the recently isolated *HelB* gene (Biswas et al., 1997a,b). This helicase was initially isolated as part of the multienzyme polymerase α complex (Biswas et al., 1993,1993a,1995). Deletion of *HCSA* does not cause nonsense suppression, demonstrating that the nonsense suppression phenotypes observed in *upf1*Δ and *mtt1*Δ strains are not simply due to deleting a group I helicase.

The Mttp1p is an RNA helicase involved in translation termination: Previous results demonstrated that the Hel1p/Mtt1p demonstrated DNA helicase activity that was stimulated by the yeast single-stranded DNA binding protein Rpa1p (Biswas et al., 1995; Bean and Matson, 1997). The results presented here demonstrated that the purified Mtt1p also shows RNA-dependent ATPase and helicase activities (Fig. 7). Thus, similar to Upf1p (Czaplinski et al., 1995), Mtt1p also demonstrates the ability to unwind both DNA and RNA duplexes.

Several lines of evidence suggest that Mtt1p is involved in translation termination The results presented here show that; 1) a *mtt1*Δ strain demonstrates a nonsense suppression phenotype (Fig. 4); 2) the Mtt1p is polysome associated (Fig. 6); 3) the Mtt1p directly interacts with the peptidyl release factor eRF3 (Fig. 5); 4) *mtt1*Δ strains demonstrate paromomycin sensitivity. If one considers that, unlike a *upf1*Δ strain, a *mtt1*Δ strain does not stabilize nonsense-containing transcripts, then the amount of nonsense suppression per RNA molecule is greater in a *mtt1*Δ strain than in a *upf1*Δ strain (Fig. 3).

A *mtt1*Δ *upf1*Δ strain demonstrates a dramatic nonsense suppression phenotype compared with a *upf1*Δ or *mtt1*Δ strain. One possibility to explain this observation is that these proteins function at the same step in translation termination and that either Mtt1p or Upf1p can partially compensate for the loss of the other factor. Inactivation of both factors, however, leads to a much higher level of nonsense suppression. An Alternative explanation is that these two factors work at different steps in the termination process. Both Mtt1p and Upf1p function in modulating the efficiency of translation termination and Upf1p acts subsequently in promoting decay of the mRNA. The synergistic increase in nonsense suppression may be a consequence of both increasing the amount of the nonsense-containing transcript and reducing the efficiency of translation termination in a *mtt1*Δ *upf1*Δ strain.

At least two RNA helicases are involved in modulating the efficiency of translation termination: It is interesting that there appears to be at least two helicases involved in modulating the efficiency of translation termination. Helicases are enzymes that unwind a nucleic acid duplexes. It has now become clear that the ability to manipulate nucleic acid duplexes by helicases is critical for every biological process in which DNA and RNA is involved. A large number of RNA helicases have been shown to be involved in post-transcriptional control mechanisms. Examples include tRNA processing, ribosomal biogenesis, splicing, transport, translation, and mRNA turnover . These RNA helicases fall into at least two families, the most prominent superfamily is the "DEAD box" helicases or superfamily group II. The superfamily group I helicases, as those shown in Fig. 2, have been shown unwind both DNA and RNA duplexes.

At present, it is not known or understood how Upf1p and Mtt1p modulate the translation termination process. The efficiency of translation termination can be affected by altering 1) the association rates of the eRFs with the ribosome, 2) the efficiency of the eRFs in promoting peptidyl hydrolysis, or 3) the rate of disassociation of the eRFs from the ribosome after translation termination has been completed. Assays to monitor these steps in the translation termination process in order to begin to understand at what step these proteins function.

The results presented here indicate that, although the translation machinery is highly precise, the growth rates of cells do not change under conditions that reduce the accuracy of this process. For example, a *mtt1*Δ *upf1*Δ strain did not demonstrate any affects on cell growth even though translation termination is less efficient in these cells. Furthermore, strains harboring the *mof4-1* allele of *UPF1* or a *upf3*Δ, which demonstrate four-fold increased programmed frameshifting efficiency and indicating a reduction of fidelity in the process of translation elongation, also do not show any growth defects (Cui et al., 1996; Ruiz-Echevarria et al., 1998).

FIG. 1A

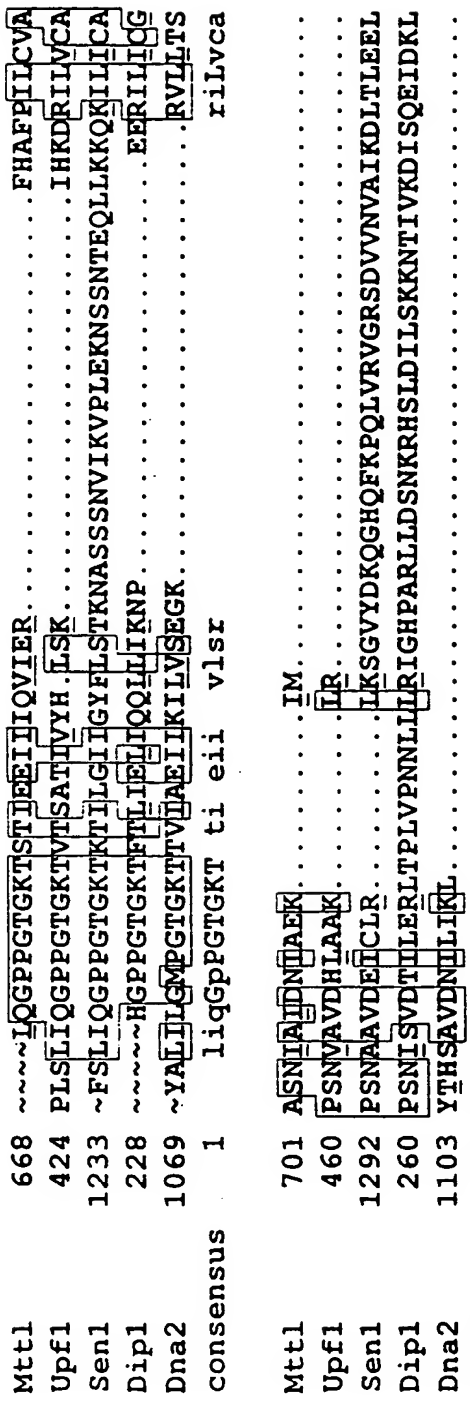

FIG. 2

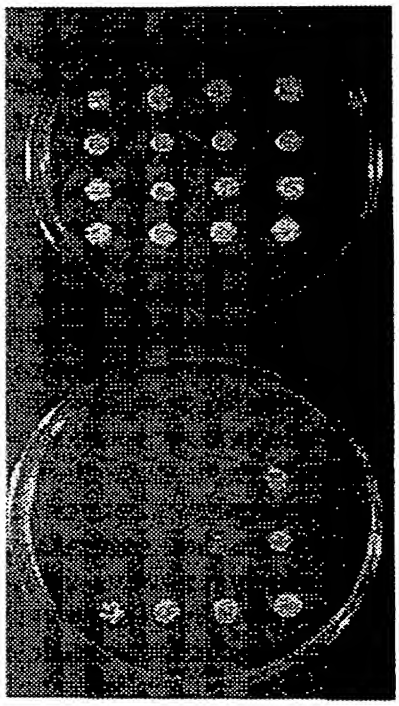
-URA
WT
upf1Δ
mtt1Δ
upf1Δ /mtt1Δ
-URA
-TRP
-TYR
WT
upf1Δ
mtt1Δ
upf1Δ /mtt1Δ
1000 100 10 0
Dilution Factor

FIG. 3

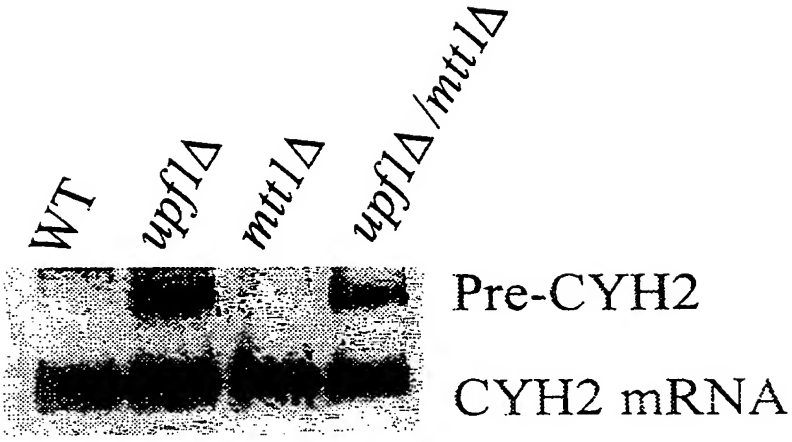

FIG. 4

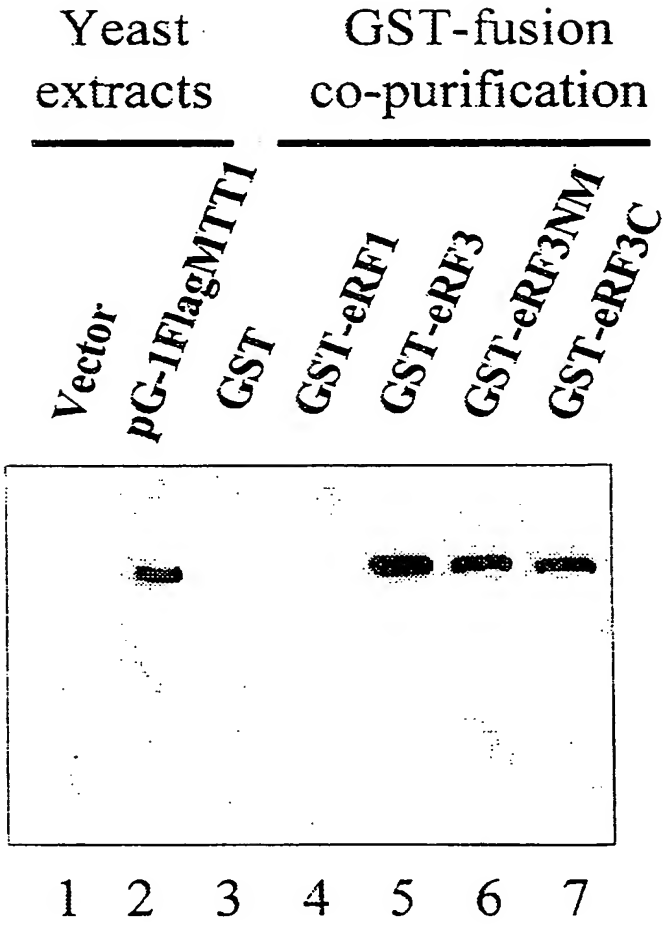
Yeast extracts
GST-fusion co-purification
Vector
pG-1FlagMTT1
GST
GST-eRF1
GST-eRF3
GST-eRF3NM
GST-eRF3C
1 2 3 4 5 6 7

FIG. 5

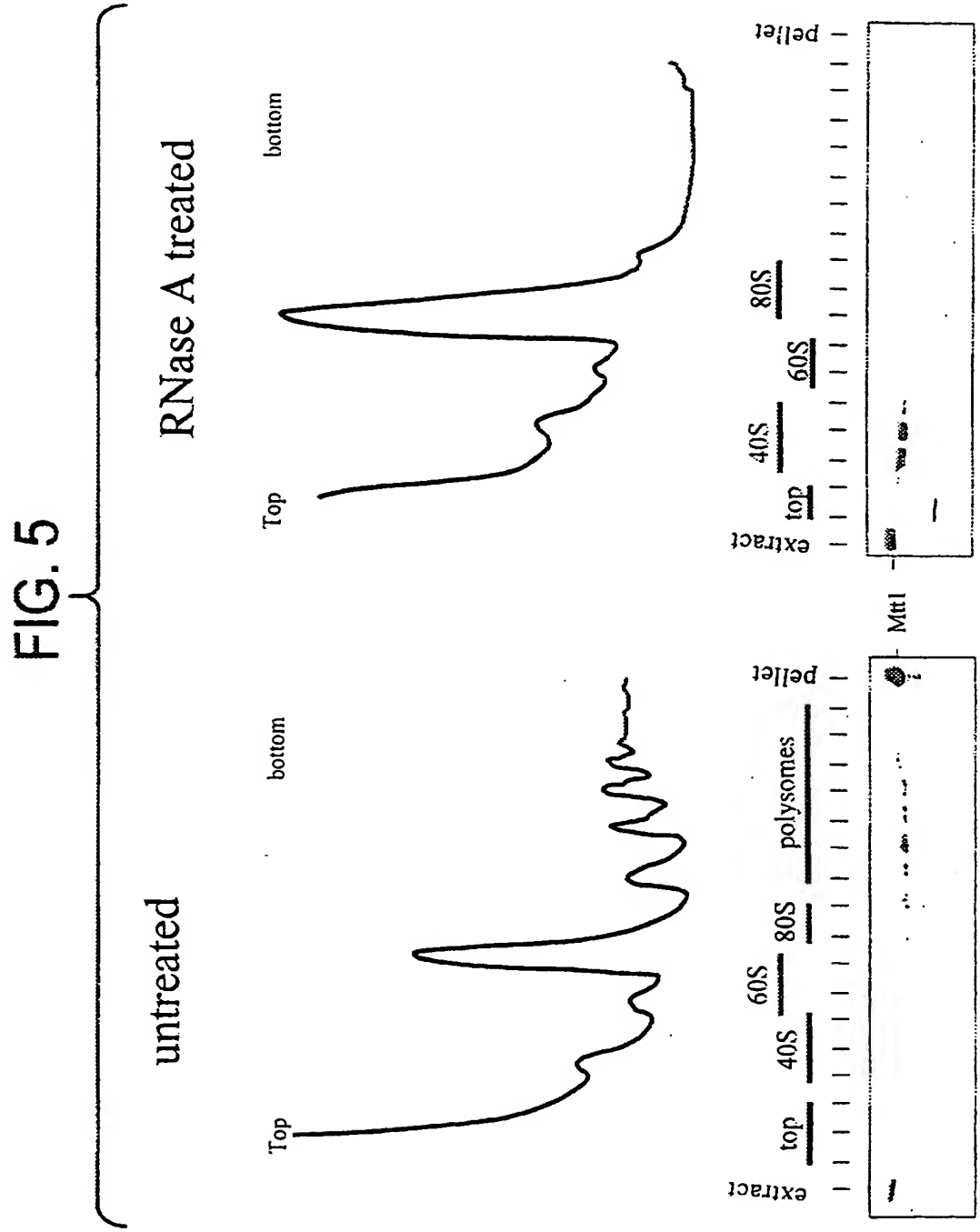
RNase A treated
untreated
bottom
Top
bottom
Top
pellet
80S
60S
40S
top
extract
pellet
polysomes
80S
60S
40S
top
extract
Mtt1

FIG. 1B

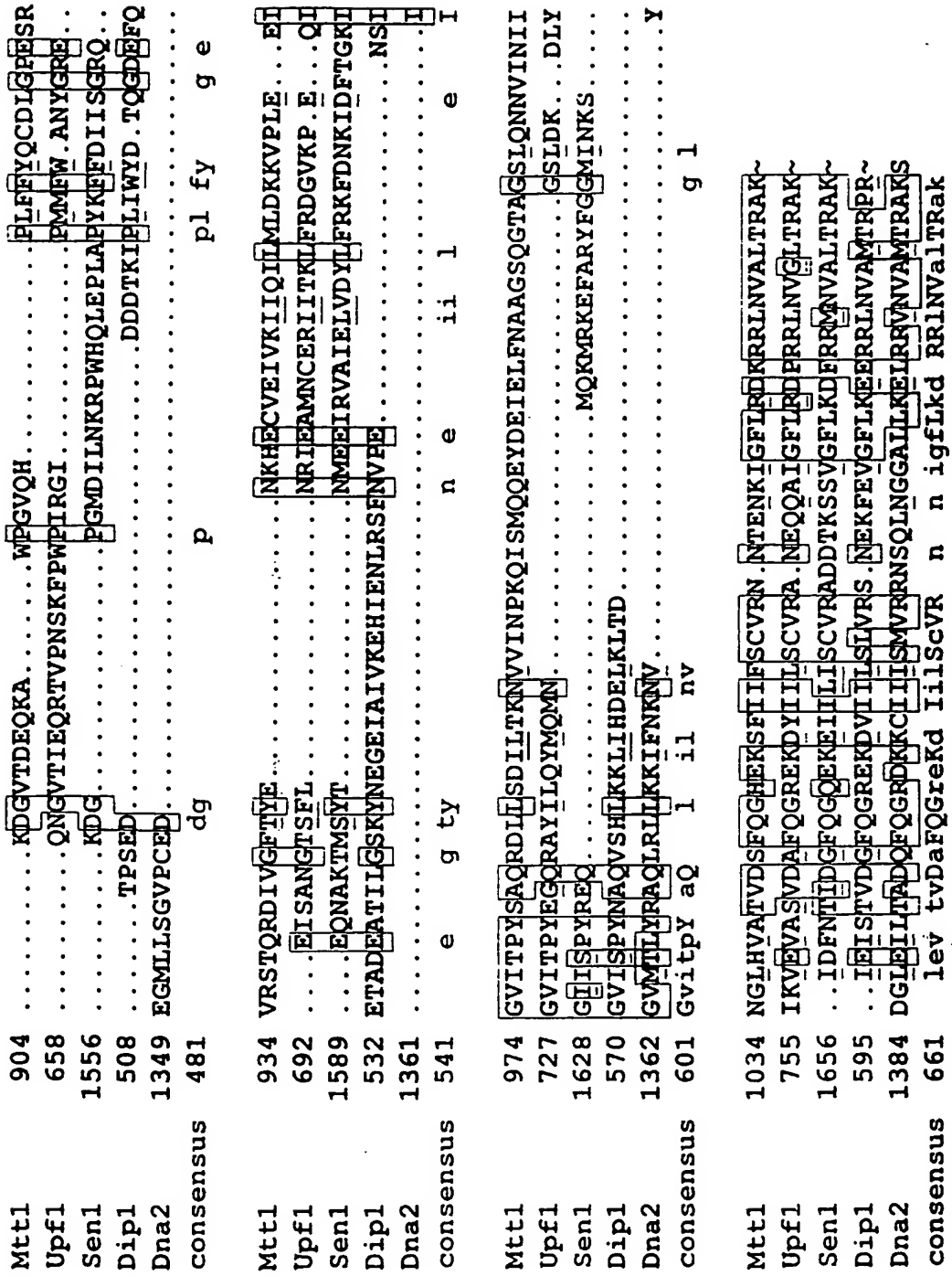
Mtt1 904 .....KDGVTDEQKA.....WPGVQH.....PLFFYQCDLGPESR
Upf1 658 .....QNGVTIEQRTVPNSKFPWPIRGI.....PMMFW.ANYGRE..
Sen1 1556 .....KDG.....PGMDILNKRPWHQLEPLAPYKKFFDIISGRQ..
Dip1 508 .....TPSED.....DDDTKIPLIWYD.TQGDEFQ
Dna2 1349 EGMLLSGVPCED.....
consensus 481 dg p pl fy g e
Mtt1 934 VRSTQRDIVGFTYE.....NKHECVEIVKIIQILMLDKKVPLE...ET
Upf1 692 ....EISANGTSFL.....NRIEAMNCERIITKLFRDGVKP.E...QI
Sen1 1589 ....EQNAKTMSYT.....NMEEIRVAIELVDYLFRKFDNKIDFTGKI
Dip1 532 ETADEATILGSKYNEGEIAIVKEHIENLRSFNVPE.....NSI
Dna2 1361 .....
consensus 541 e g ty n e ii l e I
Mtt1 974 GVITPYSAQRDLLSDILTKNVVINPKQISMQQEYDEIELFNAAGSQGTAGSLQNNVINII
Upf1 727 GVITPYEGQRAYILQYMQMN.....GSLDK...DLY
Sen1 1628 GIISPYREQ.....MQKMRKEFARYFGGMINKS.....
Dip1 570 GVISPYNAQVSHLKKLIHDELKLTD.....
Dna2 1362 GVMILYRAQLRILKKIFNKNV.....Y
consensus 601 GvitpY aQ l il nv g l
Mtt1 1034 NGLHVATVDSFQGHEKSFIIFSCVRN.NTENKIGFLRDKRRLNVALTRAK~
Upf1 755 IKVEVASVDAFQGREKDYIILSCVRA.NEQQAIGFLRDPRRLNVGLTRAK~
Sen1 1656 ..IDFNTIDGFQGQEKEIILLISCVRADDTKSSVGFLKDFRRMNVALTRAK~
Dip1 595 ..IEISTVDGFQGREKDVIILSLVRS.NEKFEVGFLKEERRLNVAMTRPR~
Dna2 1384 DGLEIITADQFQGRDKKCIIISMVRRNSQLNGGAILKEIRRYNVAMTRAKS
consensus 661 lev tvDaFQGreKd IilScVR n n igfLkd RRlNValTRak

FIG. 1A-1

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Mtt1        793 ..............................SQIIFTTNIAAGGRELKVIK.E......CP
Upf1        547 ..............................ADVVCCTCVGAGDKRLDT...K......FR
Sen1       1413 DRDEMREKNSVNYRNRDLDRRNAQAHILAVSDIICSTLSGSAHDVLATMGIK......FD
Dip1        351 ......................FKTIKDLIIQSRIVVTTLHGSSSRELCSLYRDDPNFQLFD
Dna2       1128 KHKVHPDTQKYVPNYASVKSYNDYLSKINSTSVVATTCLGI.NDILFTLNEKD.....FD
consensus   241                                l  s iv tT ig g r L ti  k       fd

Mtt1        816 VVIMDEATQSSEASTLVPLSLPGIR..NFVFVGDEKQLSSFSNIP.............QLE
Upf1        568 TVLIDESTQASEPECLIPI.VKGAK..QVILVGDHQQLG.....PVILERKAADA..GLK
Sen1       1467 TVIIDEACQCTELSSIIPLRY.GGK..RCIMVGDPNQL.....PPTVLSGAASNF..KYN
Dip1        391 TLIIDEVSQAMEPQCWIPLIAHQNQFHKLVLAGDNKQL.....PPTIKTEDDKNVIHNLE
Dna2       1182 YVILDEASQISMPVALGPLRY....GNRFIMVGDHYQL.....PPLVKNDAAR..LGGLE
consensus   301 tviiDEatQ sep  liPl   g r  r ilvGD  QL     pP i    a       le

Mtt1        862 TSLFERVLSNGTYKNPLM.LDTQYRMHPKISEFPIKKIYNGEL.................
Upf1        618 QSLFERLISLGHV..PIR.LEVQYRMNPYLSEFPSNMFYEGSL.................
Sen1       1517 QSLFVRMEKN...SSPYL.LDVQYRMHPSISKFPSSEFYQGRL.................
Dip1        446 TTLFDRIIKIFPKRDMVKFLNVQYRMNQKIMEFPSHSMYNGKLLADATVANRLLIDLPTV
Dna2       1231 ESLFKTFCEKHPES..VAELTLQYRMCGDIVTLSNFLIYDNKLKCGNNEVFAQSLELPMP
consensus   361  sLFervl       pl  LdvQYRM p isefps  iYngrL

Mtt1        904 ..............................................................
Upf1        658 ..............................................................
Sen1       1556 ..............................................................
Dip1        506 DA............................................................
Dna2       1289 EALSRYRNESANSKQWLEDILEPTRKVVFLNYDNCPDIIEQSEKDNITNHGEAELTLQCV
consensus   421
```